IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of
Junichi KAWAKAMI
Serial No. 10/541,702 Group Art Unit: 4173
Filed: January 9, 2004 Examiner: Marcos L. Sznaidman
Title: BLOOD-BRAIN BARRIER DISRUPTION INHIBITOR

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner for patents,
P. O. Box 1450
Alexandria, Virginia 22313-1450

Sir:

I, Kenji Chiba Ph.D., declare:

That I am a citizen of Japan; and my full post office address is 2-2-6, Nihonbashi-honcho, Chuo-ku, Tokyo 103-8405, Japan;

That my education and employment history is as follows:

Education

1976 April-1980 March	Department of Organic Chemistry Pharmaceutical Institute, Tohoku University
1980 April-1985 March	Department of Hygienic Chemistry Pharmaceutical Institute(Doctor course), Tohoku University

Employment

1985. April 1	Yoshitomi Pharmaceutical Industries, ltd., Researcher in Research Laboratories Tokyo
2000. April 1	Welfide Corporation Senior Principal Researcher
2001. October 1	Mitsubishi Pharma Corporation Senior Research Associate (Group manager) in Research Laboratory III (Immunology)

2002. October 1 Mitsubishi Pharma Corporation
General Manager in Research Laboratory III (Immunology)
(Head of Research Laboratory III)
2007. October 1 Mitsubishi Tanabe Pharma Corporation
General Manager in Pharmacology Laboratory
(Head of Pharmacology Laboratory)

I declare further that I have read all of the documents contained in the file wrapper of the above-entitled application.

I declare further that the test described below was conducted at my direction and under my supervision and the test results are true and correct to the best of my knowledge.

Experiments

PLP derived from rats (Peptide Institute, Inc.) was dissolved in PBS and then mixed with Freund's complete adjuvant (Sigma Chemical Co.) containing tubercule bacilli H37 Ra to prepare an emulsion. 52 of ten-weeks-old female SJL/JorllcoCrj (SJL/J) mice were immunized with each of the thus obtained emulsion of 50 μg PLP/head by subcutaneous administration of backs thereof.

An average of clinical scores of EAE (EAE score) was increased from 11 days after immunization and achieved to 2.40 $\pm$ 0.15 at 14 days after immunization, but was declined to 2.07 $\pm$ 0.13 at 17 days after immunization (Fig. 1). In this stage, the mice whose EAE scores were from 1.5 to 2.5 were pooled, and then each of 7.2 mg/4cm2 of edaravone with the patch form was applied to the backs of the mice 18 to 21 days after immunization. The obtained EAE score is shown in Fig. 2. The reapplication of edaravone patch was carried out once per 2 days. The clinical score was defined as criterions indicated in the following Table. 1. In addition, when a mouse died during the application, a score used after the death is one which was judged right before the death.

Table.1 Criterions of clinical score of experimental autoimmune encephalomyelitis (EAE)

Score	The clinical paralysis in active EAE
0	normal
0.5	stiff tail

1	limp tail
1.5	limp tail with inability to right
2	paralysis of one limb
2.5	paralysis of one limb and weakness of one other limb
3	complete paralysis of both hind limbs
4	moribund
5	death

In the control group consisting of mice to which edaravone was not administered, the average of EVE score was declined until 20 days after immunization and then was increased until 27 days after immunization, and thereafter repeat of increase and decline was shown until 39 days after immunization. In the experimental group consisting of mice where edaravone was administered, the average of EAE score remained low during the application of edaravone, as compared with the control group, in particular a significant decrease was shown 22 days after immunization or later as compared with the control group. In addition, the area under the curve (AUC) of EAE score during the administration is shown in Fig. 3. In the control group, the AUC was 38.05±4.87. In the contrast, in the edaravone-administered group, the AUC was 6.48 ±2.20 (the inhibition rate: 82.97%), which was significantly lower than that of the control group.

Furthermore, histopathological experiment to myeleterosis was carried out, when 7.2mg/4cm2 of edaravone with the patch form was applied to backs of mice during 18 to 21 days after immunization. The results are shown in Table 2.
Inspection organ: Spinal cord (Lumber part)
Immobilization/Embedding: 10% neutral-buffered-formalin fixed paraffin embedding
Staining: HE staining

Table 2

	Control			Edaravone		
	1	2	3	4	5	6
lymphocytic infiltrate	+	+	+	+	+	±
demyelinization	+	+	+	±	±	±

-: negative, ±: slight, +: mild, ++:moderate

In both the control group and the edaravone-administered group, lymphocytic

infiltrate was observed in the pia mater and the substantia alba (mainly at blood vessel circumference), and the demyelinization was observed in the bottom substantia alba of the pia mater. With respect to the degree of lymphocytic infiltrate, no clear difference was observed between both groups. The demyelinization of the edaravone administered group was lower than that of the control group to some extent.

From the above results, the edaravone administered group showed lower EAE score as compared with the control group, but as to infiltration of a lymphocyte, there is no difference between both groups. The breakdown inhibitory effect of BBB of edaravone was not confirmed. The demyelinization of the edaravone administered group was lower than that of the control group to some extent, suggesting that EAE score was kept low by inhibition of demyelinization.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent issuing thereon.

Dated of 28 February, 2008

Kenji Chiba

Kenji Chiba

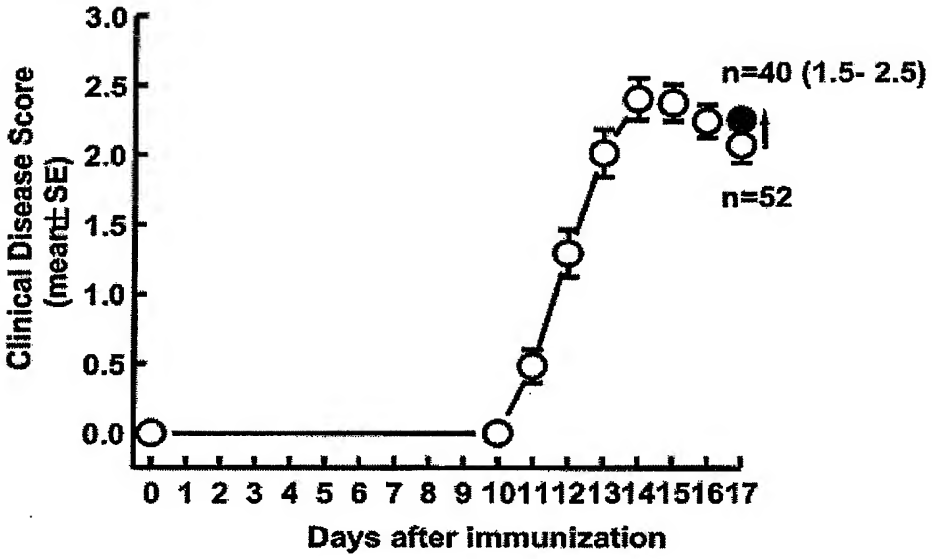

Fig. 1 Time course of clinical pathological symptoms of EAE.
EAE-developed mice with clinical disease score at 1.5 and 2.5 were selected (●), and divided into two groups consisted of twenty mice.

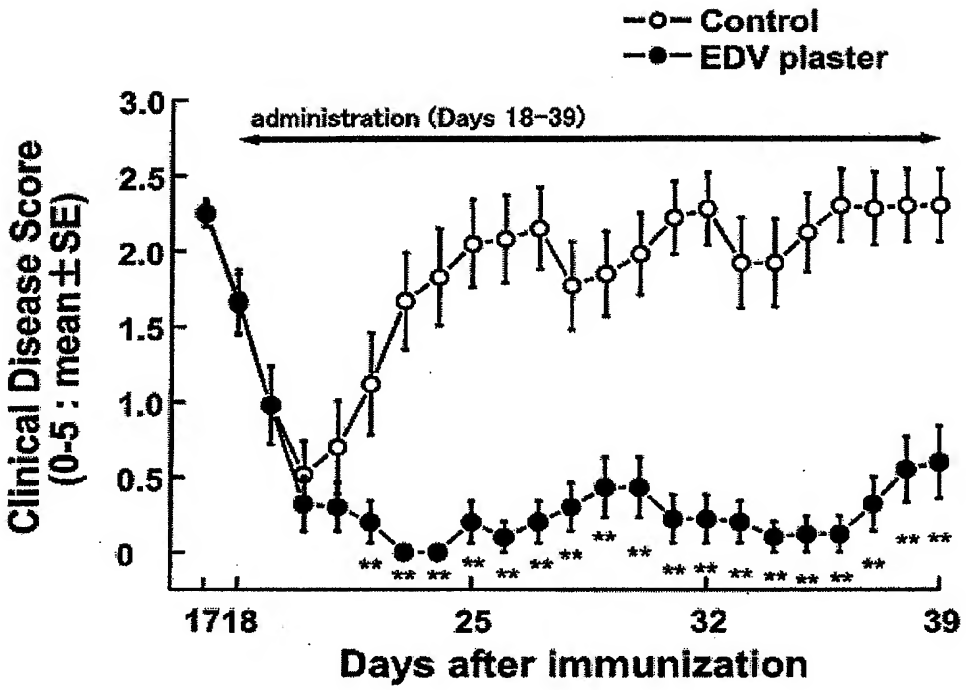

Fig. 2 Inhibition of clinical disease score in PLP induced mouse EAE by EDV plaster.
SJL/J mice was treated with EDV plaster from 18 to 39 days.
Each symbol represents the mean of the clinical disease score in 20 mice.
Statistical difference was calculated by Mann-Whitney U test as compared with vehicle.
(** : $p<0.01$)
The clinical disease score was graded as follows; 0, normal; 0.5, stiff tail; 1, limp tail;
1.5, limp tail with inability to right; 2, paralysis of one limb; 2.5, paralysis of one limb and weakness of one other limb; 3, complete paralysis of both hind limbs; 4, moribund; 5, death

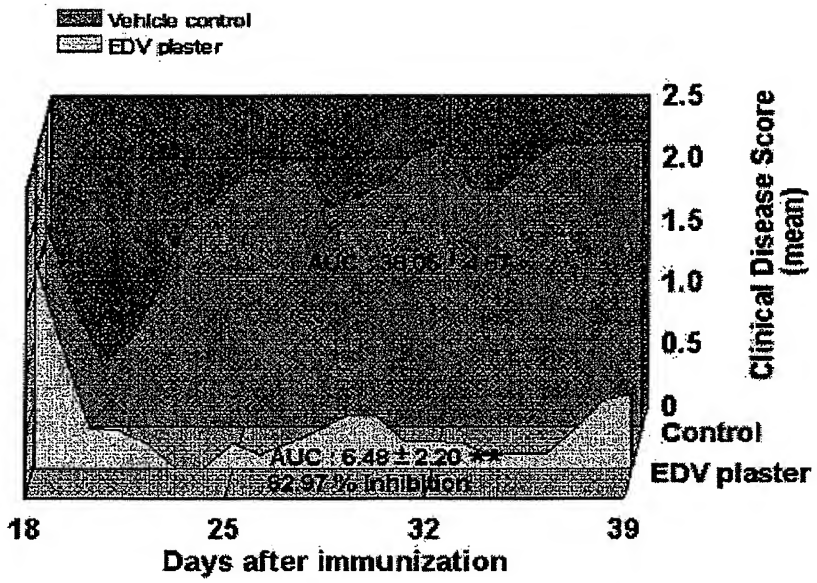

Fig. 3 AUC graph showing strong effect of EDV plaster on clinical disease score in SJL/J mice of chronic progressive EAE.

SJL/J mice was treated with EDV plaster from 18 to 39 days.

Each area represents the mean of AUC for the clinical disease score in 20 mice.

Statistical difference was calculated by *t* test as compared with control. (** : $P<0.01$)